DAKEWE CoE
Flow cytometry Training Core

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STANDARD OPERATING PROCEDURE

BD FACS Aria™III SORP Usage Instrution

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1. Policies

1.1. To standardize the daily usage of BD FACS Aria™III SORP including quality control processes, operating procedures and quality assurance measures.

2. Purpose

2.1. This file applies to all authorized personnel, including researchers, technicians, and laboratory management staff, who use the BD FACS Aria™III SORP flow cytometer in this laboratory.

3. Responsibilities

3.1. The CoE Flow Cytometry Training Core Director is responsible for instrument maintenance, periodic review of QC data, scheduling annual calibration, and personnel authorization.

3.2. User is esponsible for verifying the QC status prior to instrument operation, performing sample acquisition in accordance with this SOP, and performing post-use cleaning duties.

4. Procedures

4.1. **Starting up**

4.1.1. Register user name,sample count and durtion time in Lab book.

4.1.2. Turn on the instrument: Press the green power button on the instrument side. Allow 20 minutes for laser warm-up.

4.1.3. Log in to the computer: Username BDAdmin, Password BDIS#1

4.1.4. Open BD FACSDiva software and log in with your user profile.

4.1.5. Perform fluidics startup: In the software, select Cytometer -> Fluidics Startup and follow the on-screen prompts.

4.1.6. Insert the correct nozzle for the experiment (70µm, 85µm, 100µm, or 130µm).

4.2. Fluidics & Stream Setup

4.2.1. Ensure the sheath tank is filled with 1x PBS; empty the waste container.

4.2.2. Start the stream: Click the red "X" in the stream window. Verify the displayed nozzle size matches the one in use.

4.2.3. Check stream stability and droplet formation. The stream should be stable with a clear break-off point.

4.2.4. Activate the "Sweet Spot" for stream alignment optimization

4.3. Optics QC with CS&T Beads

4.3.1. Prepare CS&T beads: Add one drop of the current lot of BD CS&T beads (Cat#655050) to 500 µL of PBS. Vortex to mix.

4.3.2. In Diva Software:

- Select Cytometer -> CST -> View Configuration.
- Select the nozzle-specific configuration (e.g., use "85-45" for the 85µm nozzle).
- Load the bead tube into the sample chamber.
- In the Setup Control window, select Check Performance and click Run.

4.3.3. Record at least 5,000 events.

4.3.4. Acceptance Criteria:

- Three distinct peaks must be visible in all fluorescent channels.
- The brightest peak must fall within the target range.
- The %rCV for the primary detector of each laser must be ≤ 6.0.

4.4. Stream & Sort QC with Accudrop

4.4.1. Prepare Accudrop beads: Add one drop of BD Accudrop beads (Cat#345249) to 700 µL of PBS. Vortex to mix.

4.4.2. Run the Accudrop experiment located in the QC folder in Diva.

4.4.3. Adjust the drop-charge delay value until the percentage in the left stream is ≥98%.

4.4.4. Check sort precision: Must be active in "Fine Tune" mode.

4.4.5. Verify no fanning of the stream and ensure the Accudrop laser diode is properly aligned.

4.5. Post-Sort Cleanup & Shutdown

4.5.1. Routine Clean (if next user is scheduled before 5 PM on weekdays):

- 2-minute CLEAN (bleach) at flow rate 11.
- 2-minute RINSE (detergent) at flow rate 11.
- 2-minute H2O rinse at flow rate 11.
- Leave the instrument powered on.

4.5.2. Full Shutdown (if no user is scheduled after, or after 5 PM/weekends):

- Perform the routine clean steps above.
- Switch fluidics connections from the Sheath tank to the 70% EtOH tank.
- Run Fluidics Shutdown. When prompted, load a tube of 70% EtOH.
- Turn off the cytometer: First the Main Power switch, then the Laser Main switch.
- Turn off the Aerosol Management System (AMS) or Biosafety Cabinet (BSC) and the chiller.
- Important: Always log out of Diva at the end of your session for accurate usage tracking and billing.

5. Date established and subsequent reviews (annually at a minimum)

Revision	Review or Revision Date	Description of Changes	Reviewed By
1.0	01/01/25	New SOP	